

U.S.S.N 09/815,981
De Jong *et al.*
PRELIMINARY AMENDMENT

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Sub D4

centrifugation. Add 20 μ l of IdU/BrdU FITC conjugated B44 clone antibody (Becton Dickinson Immunocytometry Systems, San Jose, CA) to pellet and leave for 2 hours at room temperature in the dark with agitation every 30 minutes. Wash cells with block/permeabilization buffer and resuspend in PBS for flow analysis.

FLOW CYTOMETRY DETECTION OF FLUORESCENT IDUrd LABELED ACes

Percentage of transfected cells containing IdU labeled ACes was determined using a flow cytometry with an argon laser turned to 488 nm at 400 mW. FITC fluorescence was collected through a standard FITC 530/30-nm band pass filter. Cell populations were gated on the basis of side scatter versus forward scatter to exclude debris and doublets. Data were accumulated (15,000 events) to form bivariate channel distribution showing forward scatter versus green fluorescence (IdU-FITC). The fluorescence level at which cells were determined to be positive was established by visual inspection of the histogram of negative control cells, such that approximately 1% appeared in the positive region.

Results:

The transfection delivery results of IdU labeled ACes are set forth in Table 2.

IN THE CLAIMS:

Please replace claims 9, 10, 11, 13, 15, 16, 20, 21, and 27 with the following claims (a marked-up copy of the amended specification is attached to this Amendment):

9. (Amended) The method of claim 23, wherein step (a) comprises contacting the nucleic acid molecule with a delivery agent that comprises a cationic compound.

10. (Amended) The method of claim 9, wherein the compound is selected from the group consisting of N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine (DOPE), 2,3-

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dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanamin-
iumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$,
 $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$,
 $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$, $C_{41}H_{78}NO_8P$,
 $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$,
 $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-enyl-nonadec-10-enylenyl)
pyridinium chloride.

11. (Amended) The method of claim 1, wherein the nucleic acid
molecules are natural chromosomes, artificial chromosomes, fragments of a
chromosome or naked DNA that is greater than at least about 0.6 megabase in
size.

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13. (Amended) The method of claim 1, wherein the nucleic acid
molecules are artificial chromosome expression systems (ACes).

15. (Amended) The method of claim 14, wherein the cells are primary
cells, cell lines, plant cells, or animal cells.

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16. (Amended) The method of claim 14, wherein the cells, are stem
cells, nuclear transfer donor cells, tumor cells or transformed cells.

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20. (Amended) The method of claim 17, wherein a delivery agent
comprises a cationic compound, and the nucleic acid molecule is treated
therewith.

21. (Amended) The method of claim 20, wherein the compound is
selected from the group consisting of N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-
trimethylammonium chloride (DOTMA), dioleoylphosphatidylethanolamine
(DOPE), 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-
propanaminiumtrifluoroacetate (DOSPA), $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$,
 $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$,
 $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$,
 $C_{41}H_{78}NO_8P$, $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$,
 $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$ and (1-methyl-4-(1-octadec-9-
enyl-nonadec-10-enylenyl) pyridinium chloride.